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Mechanisms Of Osteoblast Adhesion On 3D Polymer Scaffolds Made By Rapid Prototyping

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Introduction

The use of rapid prototyping techniques for fabrication of bone tissue engineering scaffolds has been well-documented[1,2]. However, the mechanism behind their exceptional performance has not been fully explored. Since the first step of cell behavior on biomaterials is cell adhesion, this study will look at the adhesion of osteoblasts on 3D polycaprolactone (PCL) scaffolds compared to 2D PCL films.

Materials and Methods

PCL scaffolds were fabricated using a custom-designed fused deposition modeling system developed at North Dakota State University. The scaffolds had average dimensions of 5 mm × 5 mm × 2 mm. The average pore diameter was 302 µm ± 7 µm, the average strut diameter was 213 µm ± 7 µm, and the average porosity of the scaffold was 58% ± 2%. After sterilization in 70% v/v ethanol, centrifugation in sterile phosphate-buffered saline (PBS), and pre-conditioning with media, PCL scaffolds (n = 3) were seeded with 10,000 MC3T3-E1 cells. A glass ring was placed on the scaffolds to keep the total seeding area constant. For comparison, PCL films made by melt pressing (n = 3) and glass coverslips (n = 3) were sterilized, pre-conditioned, and seeded in a similar fashion. After culturing for 24 h at 37°C, all samples were immunostained for vinculin, a marker for focal adhesions[3,4]. Samples were viewed with a confocal laser scanning microscope. Images of 15 cells (average 5 cells per sample) were captured and measured for cell spread area using ImageJ 1.32j image analysis software. For 3D scaffolds, z-stack images were compressed to make one projected image. Focal adhesions were counted manually. All results were calculated as the mean ± standard error of the mean (SEM); the SEM values are the estimate of standard uncertainty. Statistical significance was found with $p < 0.05$ using a paired two-tailed T-test.

Results

Cells on glass were well-spread; vinculin staining was at the cell edges and within the cell body (Fig. 1A). Cells on PCL films were more elongated, with vinculin staining predominantly at the edges of the cells (Fig. 1B). Cells on PCL scaffolds were elongated, but smaller (Fig. 1C). Some prominent vinculin staining was at the cell edges, while faint and diffuse dot staining was evident throughout the cell body. The largest cell spread area was on glass (3777 μm^2 ± 429 μm^2), compared to PCL films (2501 μm^2 ± 310 μm^2, $p < 0.04$) and PCL scaffolds (1357 μm^2 ± 158 μm^2, $p < 0.0003$). PCL films also had a larger cell spread area compared to PCL scaffolds ($p < 0.005$). The number of focal adhesions was greatest on the glass (45 ± 4), in comparison to PCL films (25 ± 2, $p < 0.0002$) and PCL scaffolds (11 ± 1, $p < 0.0001$). The number of focal adhesions on PCL films was also greater than those on PCL scaffolds ($p < 0.0001$).

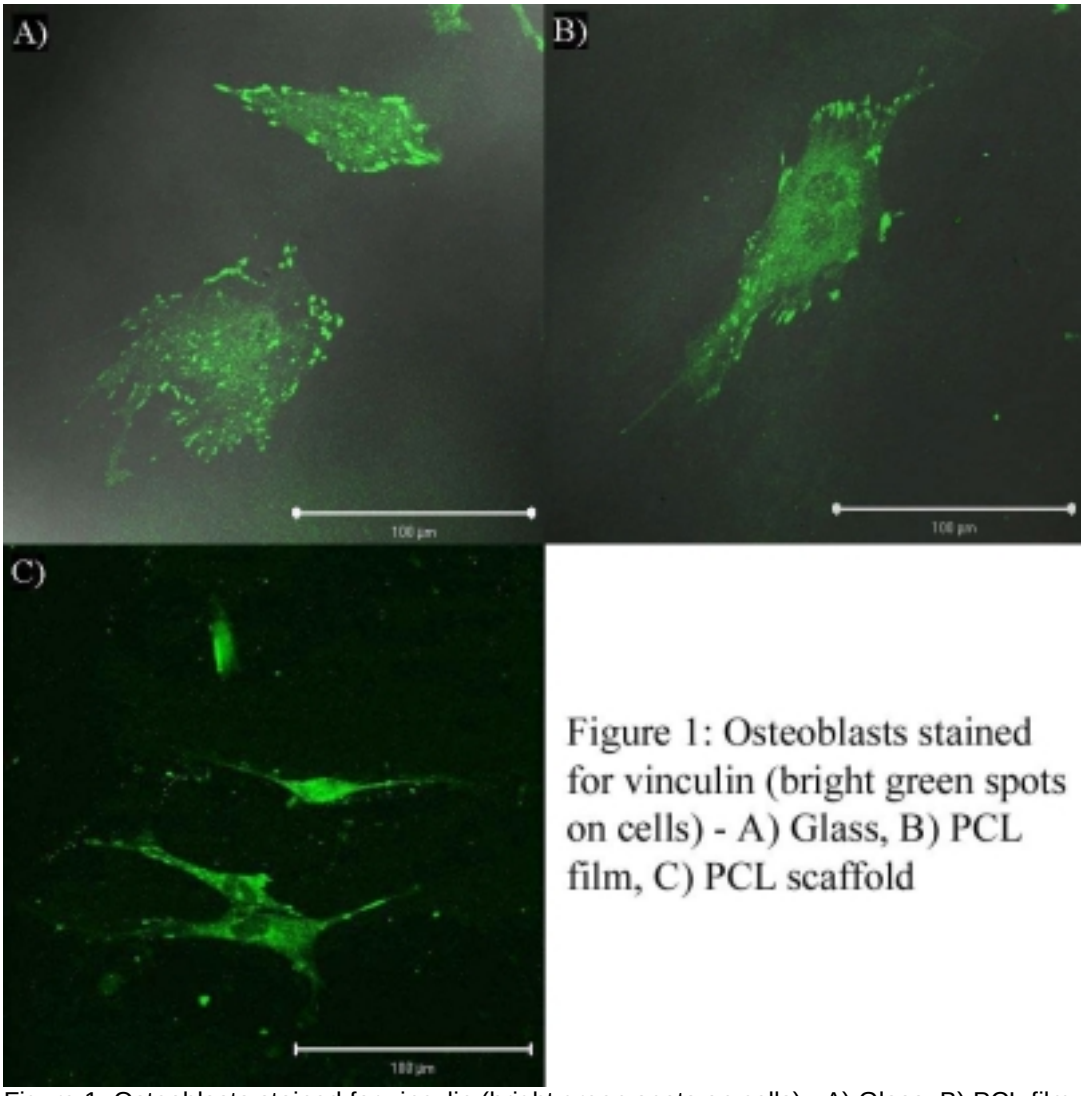

Figure 1. Osteoblasts stained for vinculin (bright green spots on cells) - A) Glass, B) PCL film, C) PCL scaffold.

Discussion

Focal adhesions can be categorized based on their size and physiological function[5]. The large focal contacts observed on 2D surfaces appeared to be well-organized, while some of the smaller focal complexes on 3D scaffolds appeared as faint dots. Large focal contacts are normally colocalized with actin stress fibers, while immature focal complexes are not associated with the actin cytoskeleton[6]. This indicates that the formation of the focal contacts on 3D scaffolds was occurring at a delayed rate compared to 2D surfaces.

The presence of growth factors is usually required to activate the proper pathways for conversion of focal complexes to focal contacts, and matrix contraction may also need to occur[7]. Consequently, this could result in a delayed rate of mature focal contact formation on these PCL scaffolds.

Actin formation has been demonstrated at later timepoints on rapid-prototyped PCL scaffolds, which promote cell proliferation and differentiation[1,2]. However, our results are at a very short timepoint. Later timepoints will need to be studied to observe focal adhesion maturation on these PCL scaffolds.

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